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Research Article

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Analysis of aroma components of dark teas from five different production regions by fully automatic headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry

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ABSTRACT

With the aim to study the volatile aroma component of dark teas from five different production regions (Yunnan, Hunan, Sichuan, Guangxi, and Hubei) in China, the fully automatic headspace solid-phase microextraction (HS-SPME) method was constructed for extracting the volatiles and gas chromatography-mass spectrometry (GC-MS) coupled with retention index (RI) of the volatiles were used to determine the volatiles variety in dark tea samples. The result showed that 105 aroma constituents were determined in five dark teas, mainly including alcohols, ketones, hydrocarbons, and methoxy-phenolic compounds, etc. Among which the methoxy-phenolic compounds were the most abundant components in the Pu-erh ripe tea, mainly including 1,2,3-trimethoxybenzene, 1,2,3-trimethoxy-5-methylbenzene, and 1,2,4-trimethoxy benzene. Ketones were the most abundant components in the Heimao tea and Yaan dark tea, mainly including β-ionone, geranyl acetone, and 6,10,14-trimethyl-2-pentadecanone. While the hydrocarbons were the most abundant components in the Liubao tea and Qingzhuan tea, mainly including 2,6,10,14-tetramethyl-pentadecane, 2,6,10,14-tetramethyl-hexadecane, and heptadecane, etc. In comparison, there were remarkable differences in flavor and aroma composition of the five types of dark tea, which may be related to the different species or subspecies, growing region and processing technology.

Key words: Different production regions, dark tea, HS-SPME, aroma components, GC-MS

INTRODUCTION

Chinese dark teas (CDTs) are post-fermented teas, one of the six major teas in China. CDTs are unique microbial fermented teas characterized by a period of fungal growth during its manufacturing process [1]. From the studies of CDTs, the microorganisms involved in the postfermentation process have been proved to be an important factor in forming the quality and flavor of CDTs [2]. Various CDTs are traditionally further divided into Yunnan Pu-erh ripe tea, Hunan Heimao tea, Sichuan Yaan dark tea, Guangxi Liubao tea, and Hubei Qingzhuan tea, according to the different producing areas and processing technologies employed [3]. The discovery of special beneficial physiological effect of CDTs has attracted more and more attention worldwide as a hot topic in the recent field of tea science [4,5].

Volatile aroma components of tea are one of the most important factors affecting the character and quality of tea [6]. The characterization aroma components in different types of tea have been described in many studies [7-10], using different extraction methods, such as simultaneous distillation and extraction (SDE) and vacuum distillation extraction (VDE). However, both of these two methods are hazardous because of required amounts of toxic and expensive solvents, and can not reveal the whole spectrum of volatile compounds [11]. Fully automatic headspace-solid phase microextraction (HS-SPME) technique was performed in the present study to analyze the different dark tea samples, for its advantages of rapid, simple, convenient, and solvent-free [12]. In recent years, this method has been widely used for determining the volatile aroma components of various foods, showing good repeatability, sensibility and selectivity [13-15].

Till date, no studies have been reported about the characterization of CDTs using GC-MS. For this purpose, HS-SPME/GC-MS was applied to identify and quantify the aroma components of CDTs from five different producing areas. Our results will shed new light on CDTs production and provide a scientific guiding role on the special flavour quality assessment of CDTs.

EXPERIMENTAL SECTION

Tea samples

In this work, 5 dark tea samples collected from five different dark tea producing areas (Yunnan, Hunan, Sichuan, Guangxi, and Hubei) in China, there were Yunnan Pu-erh ripe tea, Hunan Heimao tea, Sichuan Yaan dark tea, Guangxi Liubao tea, and Hubei Qingzhuan tea. All collected tea samples were picked and processed in 2012. Since geographical discrimination was attempted, the production areas were shown in Fig.1.

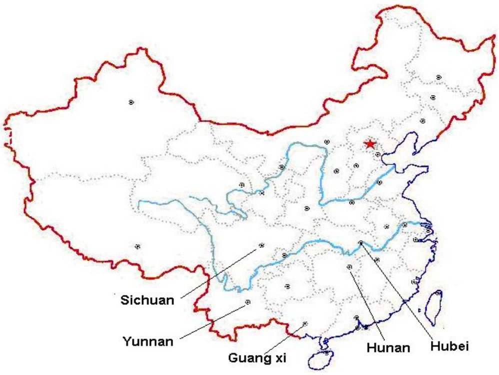


Fig.1: The map of distribution areas of CDTs

HS-SPME analysis

The fiber coatings 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) were purchased from Supelco (Bellefonte, PA). The fiber used for analysis was previously kept into the GC-MS injection port for 30 min at 250 °C.

The extraction procedure and chromatographic conditions were developed and validated in our previous studies [16]. All of the tea samples were ground to pass through 30-60 mesh. The ground tea sample (2.0 g) was introduced into a 20 mL sealed headspace bottle, and infused with 5 mL boiling water, and the temperature of headspace bottle was kept at 80 °C with a hot plate. Then, an SPME fiber was exposed to the headspace of the sample while the tea powder was continuously stirred (250 rpm) for 60 min. All of the volatile compounds absorbed on the SPME fiber were desorbed at GC-MS injection port (250 °C for 3.5 min), and followed analyzed by GC-MS, separately.

GC-MS analysis

Agilent 7890A GC system, coupled with Agilent 5975C mass selective detector (MSD) (Agilent, Santa Clara, CA) were used to identify volatile compounds of all the tea samples. An Agilent HP-5MS capillary column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness) was equipped and the helium (percentage purity > 99.999%) flow rate was 1 mL/min. The injector temperature was 250 °C and the injection mode was splitless. The GC oven temperature was held at 50 °C for 5 min, and programmed from 50 °C to 210 °C at 3 °C/min, this temperature was held for 3 min and then increased to 230 °C at 15 °C/min. The mass spectrometer was operated in an electron-impact mode of 70 eV with a source temperature of 230 °C and a quadrupole set of 150 °C, the mass scan range was 30-500 atomic mass units (amu), and solvent delay time was 2.8 min.

Data analysis

Identifications of the peaks were made by searching National Institute of Standards and Technology (NIST) 08.L MS library (a match quality of 95% minimum was used as a criterion) and their retention indices (RI) were compared with the published data [12,17].

The relative percentages of the detected peaks were obtained by peak-area normalization and all relative response factors being taken as one. In order to get the Kovats RI for each peak, 1 µL n-alkane mixture (C8-C40; Sigma-Aldrich, USA) was injected under the same GC-MS conditions. Kovats RI Indices were derived using the classical formula of Kovats:

$$\mathrm{RI} = 100n + \frac{100\,[\log(t_x) - \log(t_n)]}{\log(t_{n+1}) - \log(t_n)}$$

Where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound “x”, t_x is the retention time of compound “x”.

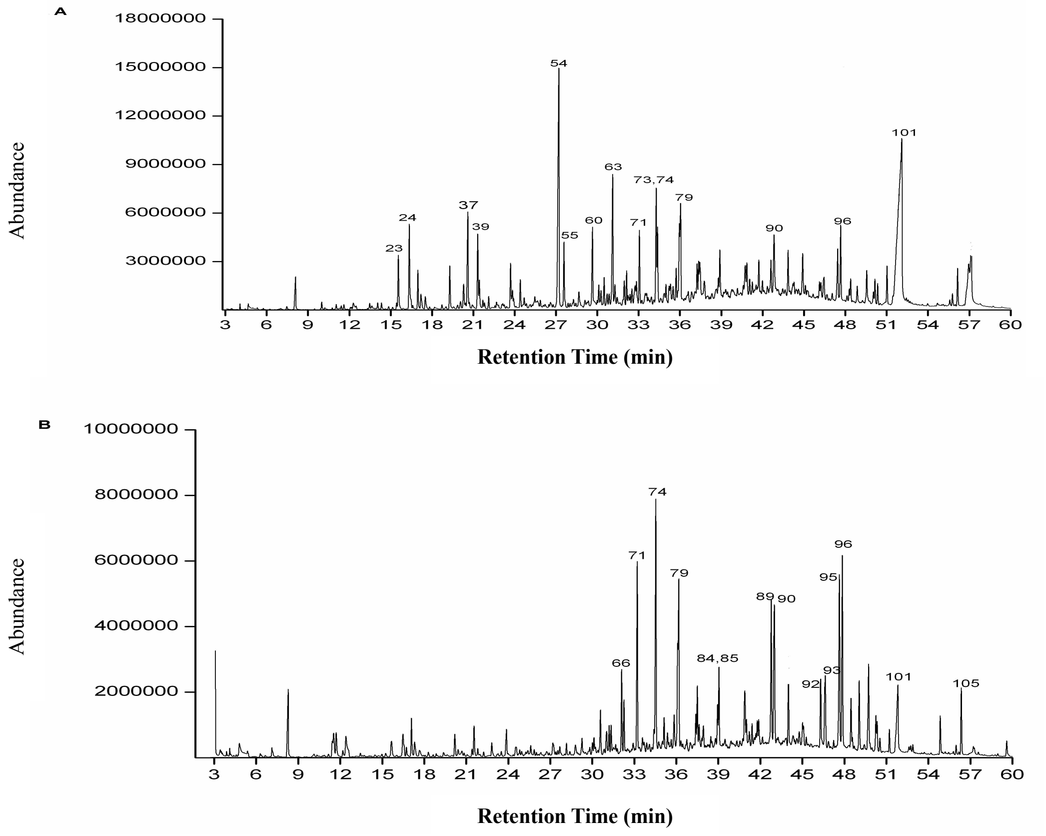
RESULTS

Analysis of volatile compounds in five dark tea samples

In TIC of tested tea samples, the identification of peaks was carried out by NIST searching. Altogether 105 major aroma compounds were identified in five dark tea samples, and the information of these compounds was shown in Table 1. The typical total ion chromatograms (TIC) were presented in Fig. 2. These volatile compounds with relative percentage content ≥ 2.00% in five dark tea samples were denoted by the numbers shown in Table 1.

The aroma compounds identified by GC-MS in five dark tea samples covered ten categories of compounds in the classification of organic chemistry. These compounds included methoxy-phenolic compounds, alcohols, hydrocarbons, esters, acids, aldehydes, ketones, lactones, and nitrogen compounds, etc. A total of 73 aroma compounds were identified in Pu-erh ripe tea, and jointly represented of 95.64% of the headspace extracts. These identified compounds mainly including 1,2,3-trimethoxybenzene (12.58%), linalool oxides (10.15%), hexadecanoic acid (6.35%), 1,2,3-trimethoxy-5-methylbenzene (4.85%), alpha-iso-methylionone (4.26%), dihydroactinidiolide (3.28%), α-terpineol (2.77%), and 1,2,4-trimethoxybenzene (2.71%), etc.

A total of 68 aroma compounds were identified in Heimao tea, and jointly represented of 95.34% of the headspace extracts. These identified compounds mainly including β-ionone (9.12%), 6,10,14-trimethyl-2-pentadecanone (6.91%), caffeine (6.64%), 2,6,10,14-tetramethyl-pentadecane (6.62%), geranyl acetone (6.44%), dihydroactinidiolide (5.79%), heptadecane (4.99%), hexadecanoic acid (4.46%), and 2,6,10,14-tetramethyl -hexadecane (3.71%), etc.



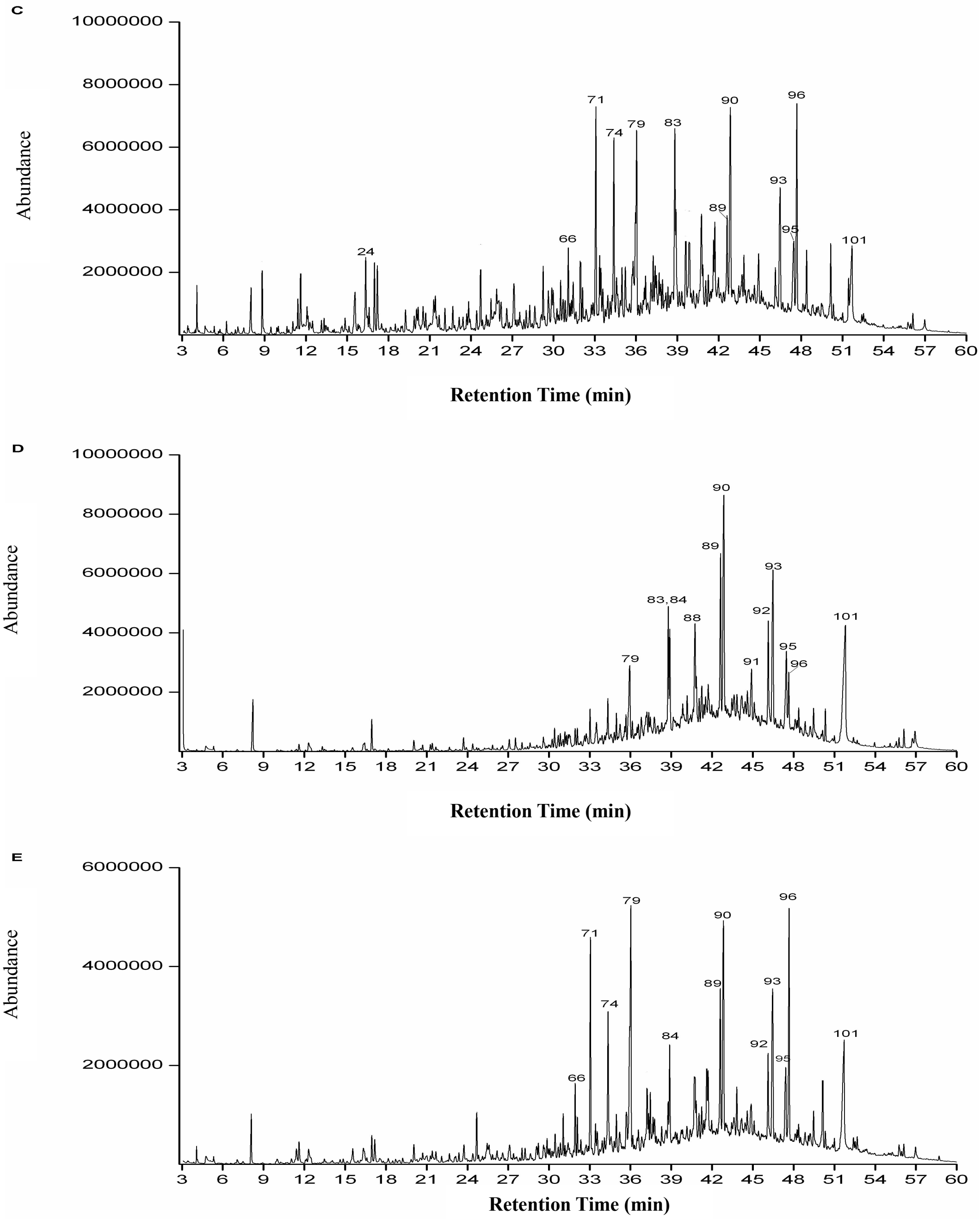


Fig.2 GC-MS TIC of aroma components in the five dark teas (A: Pu-erh ripe tea, B: Heimao tea, C: Yaan dark tea, D: Liubao tea, and E: Qingzhuan tea), these components with relative percentage content ≥ 2.00% in five dark tea samples were denoted by the numbers shown in Table 1

Table 1: GC-MS analysis results of aroma components in the five kinds of dark tea

NO.	RI^a	Compound^b	I.D.^{c}	Relative percentage content (%)				
				Pu-erh ripe tea	Heimao tea	Yaan dark tea	Liubao tea	Qingzhuan tea
1	806	Hexanal	RI,MS	0.13	0.16	0.73	—	0.32
2	861	Hexyl alcohol	RI,MS	—	0.12	0.29	—	—
3	884	2-Heptanone	RI,MS	—	0.24	0.22	0.10	0.18
4	893	Heptanal	RI,MS	0.14	—	0.10	—	0.08
5	927	*α*-Pinene	RI,MS	—	—	1.63	—	—
6	942	Camphene	MS	—	—	0.20	—	—
7	951	6-Methyl-2-heptanone	MS	—	—	0.15	—	—
8	957	Benzaldehyde	RI,MS	0.25	0.17	0.15	0.12	0.21
9	968	1-Heptanol	RI,MS	—	—	0.15	—	—
10	979	1-Octen-3-ol	RI,MS	0.14	0.13	0.28	—	0.23
11	985	6-Methyl-5-hepten-2-one	RI,MS	0.11	0.87	0.62	0.12	0.45
12	989	*β*-Pinene	RI,MS	0.17	1.09	1.70	0.38	0.79
13	996	2-Pentyl-furan	RI,MS	—	0.32	0.33	0.08	0.11
14	998	Octanal	RI,MS	—	—	0.20	—	—
15	1005	(E,E)-2,4-Heptadienal	MS	—	—	0.24	—	—
16	1020	Isopropyl benzene	RI,MS	—	—	0.29	—	—
17	1026	*D*-Limonene	RI,MS	—	0.13	0.31	0.23	—
18	1030	2-Ethylhexanol	MS	0.22	—	—	0.10	0.14
19	1032	Benzyl alcohol	RI,MS	0.14	—	0.12	—	—
20	1040	Phenyl acetaldehyde	RI,MS	0.21	—	—	—	0.09
21	1048	1-Ethyl-1H-pyrrole-2- carbaldehyde	MS	0.24	0.11	—	—	—
22	1064	Acetophenone	RI,MS	0.09	0.21	0.19	—	—
23	1072	Linalool oxide I	RI,MS	2.13	1.02	1.55	0.33	0.78
24	1087	Linalool oxide II	RI,MS	3.29	1.09	2.03	0.41	0.64
25	1092	*(E,E)*-3,5-Octadien-2-one	RI,MS	0.09	0.32	0.44	0.07	0.11
26	1098	Linalool	RI,MS	1.32	1.36	1.69	1.47	0.88
27	1104	Nonanal	RI,MS	0.76	—	1.70	0.16	0.89
28	1110	Phenylethyl alcohol	RI,MS	0.62	0.41	0.16	—	0.28
29	1122	Campholenic aldehyde	MS	—	—	0.21	—	—
30	1134	2,5-Pyrrolidinedione, 1-ethyl-	RI,MS	0.17	—	0.12	—	0.16
31	1139	3-Nonene-2-ketone	RI,MS	—	—	0.13	—	—
32	1141	4-Oxoisophorone	RI,MS	0.10	—	0.18	—	0.22
33	1149	1,2-Dimethoxybenzene	RI,MS	1.61	0.20	0.63	0.09	0.19
34	1159	*(E)*-2-Nonenal	RI,MS	—	—	0.30	—	—
35	1164	1,3-Dimethoxybenzene	MS	—	—	0.41	—	—
36	1169	Linalool oxide III	RI,MS	1.00	0.21	—	—	—
37	1175	Linalool oxide IV	RI,MS	3.73	0.27	0.60	0.17	—
38	1178	Naphthalene	RI,MS	0.16	0.14	0.44	0.30	0.22
39	1188	*α*-Terpineol	RI,MS	2.77	0.25	0.28	0.31	0.15
40	1190	Methyl salicylate	RI,MS	0.98	1.12	0.50	0.40	0.24
41	1196	Safranal	RI,MS	0.24	0.20	0.36	0.14	0.28
42	1205	Decanal	RI,MS	0.42	0.18	0.59	—	0.20
43	1218	*β*-Cyclocitral	RI,MS	0.20	0.60	0.55	0.15	0.26
44	1224	2-Carene	MS	—	—	—	—	0.12
45	1228	Nerol	RI,MS	—	—	—	—	0.21
46	1241	3,4-Dimethoxytoluene	RI,MS	1.59	—	0.34	—	—
47	1256	Geraniol	RI,MS	0.98	0.62	0.52	0.33	0.33
48	1260	*(E)*-2-Decanal	MS	—	—	1.83	0.11	1.77
49	1269	Citral	RI,MS	—	—	0.24	—	—
50	1287	2-Methyl-naphthalene	RI,MS	0.20	0.21	0.69	0.20	0.14
51	1294	2-Undecanone	RI,MS	—	—	0.88	—	0.33
52	1300	Tridecane	RI,MS	—	—	—	0.10	—
53	1302	1-Methyl-naphthalene	RI,MS	0.27	0.22	0.64	0.22	0.37
54	1316	1,2,3-Trimethoxybenzene	RI,MS	12.58	0.73	1.43	0.43	0.62
55	1325	4-Ethyl-1,2-dimethoxy- benzene	RI,MS	2.38	0.22	0.21	0.54	—
56	1334	2,6,6-Trimethyl-1-cyclohexene-1-ethanol	MS	0.12	—	0.23	—	—
57	1351	2,6-Dimethoxyphenol	RI,MS	0.67	0.46	0.60	—	—
58	1359	*γ*-Nonanolactone	MS	—	0.65	—	—	—
59	1362	2-Undecenal	RI,MS	—	—	1.14	—	0.46
60	1375	1,2,4-Trimethoxybenzene	RI,MS	2.71	—	—	—	0.32
61	1397	*cis*-Jasmone	RI,MS	0.56	0.27	0.74	0.68	0.49
62	1400	Tetradecane	RI,MS	0.39	0.92	0.46	0.56	0.45
63	1406	1,2,3-Trimethoxy-5- methyl-benzene	RI,MS	4.85	0.80	1.58	0.25	1.17
64	1409	*α*-Cedrene	RI,MS	0.66	0.87	—	0.69	0.28
65	1417	*β*-Caryophyllene	RI,MS	—	0.19	—	—	—
66	1428	*α*-Ionone	RI,MS	0.80	2.71	2.92	0.77	2.15
67	1438	Dihydro-*β*-ionone	MS	0.36	—	0.30	0.33	—

NO.	RI[a]	Compound[b]	I.D.[c]	Relative percentage content (%)				
				Pu-erh ripe tea	Heimao tea	Yaan dark tea	Liubao tea	Qingzhuan tea
68	1442	1-Methoxy-naphthalene	RI,MS	0.50	—	—	—	—
69	1447	2-Methoxy-naphthalene	RI,MS	0.93	—	—	0.29	—
70	1449	1,2,3,4-Tetramethoxy- benzene	RI,MS	0.59	0.29	0.44	0.40	—
71	1455	Geranyl acetone	RI,MS	2.45	6.44	5.38	1.46	6.53
72	1468	5-Methoxy-6,7-dimethyl- benzofuran	RI,MS	—	—	0.89	—	0.92
73	1480	alpha-Iso-methylionone	MS	4.26	0.57	0.43	0.36	—
74	1487	*β*-Ionone	RI,MS	2.26	9.12	4.44	1.73	4.65
75	1492	2-Tridecanone	MS	—	—	—	0.76	—
76	1500	Pentadecane	RI,MS	0.63	0.96	1.16	1.05	1.18
77	1506	Dibenzofuran	RI,MS	0.65	0.38	1.17	1.18	0.63
78	1510	*α*-Farnesene	RI,MS	0.65	—	—	—	—
79	1528	Dihydroactinidiolide	RI,MS	3.28	5.79	4.66	6.07	7.70
80	1554	Nerolidol	RI,MS	1.32	0.85	0.50	0.42	1.24
81	1572	Fluorene	MS	1.10	0.85	0.65	1.05	—
82	1576	Spathulenol	MS	—	—	0.63	—	—
83	1598	Cedrol	RI,MS	0.51	1.25	4.59	5.25	1.05
84	1600	Hexadecane	RI,MS	1.58	2.50	1.73	4.34	2.94
85	1649	Methyl jasmonate	RI,MS	—	2.05	—	—	—
86	1653	*α*-Cadinol	RI,MS	1.20	0.74	0.97	1.93	1.02
87	1659	2,2',5,5'-Tetramethyl-1,1'- biphenyl	RI,MS	0.61	0.51	0.69	1.19	0.97
88	1664	2-Methyl-hexadecane	RI,MS	0.46	0.61	0.85	2.33	1.19
89	1700	Heptadecane	RI,MS	1.12	4.99	2.09	7.58	4.47
90	1706	2,6,10,14-Tetramethyl- pentadecane	RI,MS	2.49	6.62	6.79	14.75	9.34
91	1765	Anthracene	RI,MS	1.71	0.63	1.67	3.16	1.75
92	1800	Octadecane	RI,MS	0.45	2.94	1.03	4.05	2.26
93	1809	2,6,10,14-Tetramethyl- hexadecane	RI,MS	1.22	3.71	4.45	10.48	7.11
94	1828	Isopropyl myristate	RI,MS	—	0.29	—	—	—
95	1840	Caffeine	MS	1.95	6.64	3.04	5.64	3.12
96	1846	6,10,14-Trimethyl-2- pentadecanone	RI,MS	2.60	6.91	5.80	2.36	7.92
97	1900	Nonadecane	RI,MS	—	—	—	1.33	0.92
98	1918	Farnesyl acetone	RI,MS	0.45	1.21	—	—	—
99	1927	Hexadecanoic acid- methyl ester	RI,MS	0.72	0.53	0.45	1.44	0.63
100	1949	Isophytol	RI,MS	1.31	0.76	0.23	—	0.44
101	1975	Hexadecanoic acid	RI,MS	6.35	4.46	2.96	3.87	7.32
102	2000	Eicosane	RI,MS	—	0.28	—	0.16	0.41
103	2093	Methyl linoleate	RI,MS	—	—	—	0.27	0.49
104	2099	Methyl linolenate	RI,MS	0.43	0.32	0.22	0.41	0.14
105	2122	Phytol	RI,MS	1.31	2.35	0.42	0.86	0.43

"—" not found. [a] *RI, Retention indices as determined on HP-5MS column using the homologous series of n-alkanes (C_8–C_{40}).* [b] *Compounds are listed in order of retention time.* [c] *Method of identification: MS, identified by comparison with mass spectra; RI, identified by retention indices.*

A total of 84 aroma compounds were identified in Yaan dark tea, and jointly represented of 93.85% of the headspace extracts. These identified compounds mainly including 2,6,10,14-tetramethyl-pentadecane (6.79%), 6,10,14-trimethyl-2-pentadecanone (5.80%), geranyl acetone (5.38%), dihydroactinidiolide (4.66%), cedrol (4.59%), 2,6,10,14-tetramethyl-hexadecane (4.45%), *β*-ionone (4.44%), caffeine (3.04%), hexadecanoic acid (2.96%), and *α*-ionone (2.92%), etc.

A total of 62 aroma compounds were identified in Liubao tea, and jointly represented of 96.43% of the headspace extracts. These identified compounds mainly including 2,6,10,14-tetramethyl-pentadecane (14.75%), 2,6,10,14-tetramethyl-hexadecane (10.48%), heptadecane (7.58%), dihydroactinidiolide (6.07%), caffeine (5.64%), cedrol (5.25%), hexadecane (4.34%), octadecane (4.05%), hexadecanoic acid (3.87%), and anthracene (3.16%), etc.

A total of 67 aroma compounds were identified in Qingzhuan tea, and jointly represented of 94.08% of the headspace extracts. These identified compounds mainly including 2,6,10,14-tetramethyl-pentadecane (9.34%), 6,10,14-trimethyl-2-pentadecanone (7.92%), dihydroactinidiolide (7.70%), hexadecanoic acid (7.32%), 2,6,10,14-tetramethyl-hexadecane (7.11%), geranyl acetone (6.53%), *β*-ionone (4.65%), heptadecane (4.47%), caffeine (3.12%), hexadecane (2.94%), and octadecane (2.26%), etc.

It was observed that there were considerable amounts of methoxy-phenolic compounds such as 1,2,3-trimethoxy benzene, 1,2,3-trimethoxy-5-methylbenzene and 1,2,4-trimethoxy benzene in the Pu-erh ripe tea, these compounds were considered to be the product of the degradation and methylization of the tea catechins by the microorganisms during the post-fermentation [5]. There were large amounts of ketones existed in Heimao tea, Yaan dark tea, and Qingzhuan tea, such as *β*-ionone, geranyl acetone, and *α*-ionone, these compounds were probably derived from the oxidation and condensation of carotenoids [18] , there were very important aroma components in these teas.

Hydrocarbons were the most abundant components in Liubao tea, such as 2,6,10,14-tetramethyl-pentadecane, 2,6,10,14-tetramethyl-hexadecane and heptadecane, these components had poor volatility, thus had no effect to the flavor of the Liubao tea [19]. In all, these differences among the dark teas may relate to the different species or subspecies, growing seasons, geographical regions, processing, and fermentation methods.

Analysis of chemical differences of aroma compounds in five dark teas

The aroma compounds comparison result in 5 different dark teas was shown in Fig.3. As shown in Fig.3, there were great differences in the content of aroma components among CDTs. The methoxy-phenolic compounds were the most abundant aromatic components in the Pu-erh ripe tea (28.39%), its content in other teas were less than 7.10%. The ketones were the major aromatic components in Heimao tea (28.87%), Yaan dark tea (22.82%), and Qingzhuan tea (22.03%), while Pu-erh ripe tea and Liubao tea were only detected 14.13% and 8.74%, respectively. In addition, except for Pu-erh ripe tea, the hydrocarbons accounted for a large proportion in other CDTs, especially in the Liubao tea (54.15%). The content of esters, aldehydes, lactones and nitrogen compounds were relatively lower in all the dark tea samples.

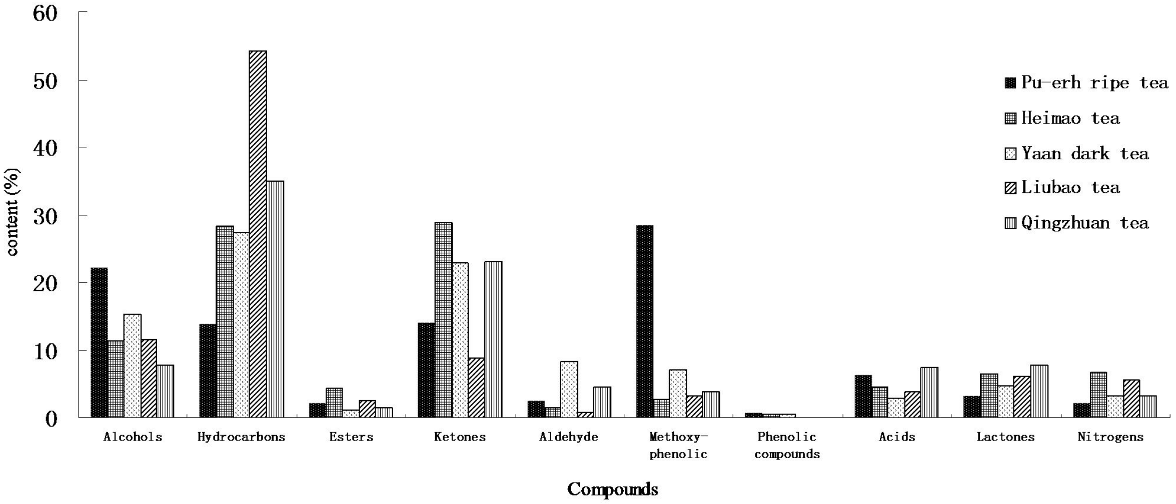


Fig.3 Comparison of aroma components in five kinds of dark tea

CONCLUSION AND DISCUSSION

In this work, a fully automatic HS-SPME/GC–MS method was developed for the determination of the aroma components of CDTs from five different producing areas. Results showed that 105 major aroma compounds were identified, and there were great differences in the content of aroma components in these teas. The methoxy-phenolic compounds (28.39%) were potentially responsible for the special characteristic aroma components in Pu-erh ripe tea, and the hydrocarbons (54.05%) were the most abundant characteristic aroma components in Liubao tea, while ketones were the major aromatic components in Heimao tea (28.87%), Yaan dark tea (22.82%), and Qingzhuan tea (22.03%), respectively. These differences may be related to the different producing region and processing technology of dark tea. Meanwhile, some of the more advanced instruments such as gas chromatography-olfactometry (GC-O) should be used for the detection and identification of crucial and characteristic aromatic compounds, and then explore the relationship between these odor compounds and the quality of dark tea and establish a more scientific and practical model to evaluate the quality of CDTs.

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